EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF ORGANS IN THE EMBRYO OF THE FOWL (GALLUS DOMESTICUS).

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II. The Development of Defective Embryos, and the Power of Regeneration.[1]

Born has shown that young embryos of the frog possess immense power of healing smoothly cut wounds, and that the vitality of even small isolated parts is remarkable ; pieces of the head or of the tail, for instance, may continue to grow and develop in spite of the complete absence of the heart, blood and blood vessels, and nervous system, for a period of about three weeks, or until the yolk contained in the cells is fully consumed. "The development of each organ progresses as far as the cut surface as well as in the normal larva, no matter what the position of the cut surface may be ; the absence of the heart or the brain does not affect the subsequent processes of growth and differentiation in any marked way."

These conclusions are of great importance for the physiology of development. So far as I know, similar experiments have not been performed on the embryos of *Amniota*, and the following account may serve to fill up the gap in part. Striking differences appear between the embryo of the fowl and of the frog in regard to the vitality of defective embryos, or of parts of embryos : The chick embryo dies very rapidly after destruction of the heart, for the embryonic tissues are dependent to an extreme degree upon the circulation ; it therefore follows that small parts of an embryo cannot undergo differentiation as in the frog. On the other hand, the growth of the extra-embryonic blastoderm is independent of blood supply, and may continue in eggs in which there is no embryo owing to destruction of the heart, until nearly the entire yolk is covered. This difference between the embryo of the chick and its own extra-embryonic blastoderm or the embryo of

[1] The first part appeared in Biol. Bull., 1903, Vol. V., No. 2 : "Experiments on the Amnion and the Production of Anamniote Embryos."

the frog, may be explained by the simple consideration that the cells of the embryonic tissues proper in the chick are devoid of yolk or other nutriment, and hence are dependent for their subsequent growth and differentiation upon the circulation ; whereas the cells of the frog embryo are loaded with food in the form of yolk ; and the extra-embryonic blastoderm of the chick is a digestive organ lying on an immense reservoir of food.

The necessity of circulation for all normal development of the chick embryo beyond the stage of about 33 hours (12–14 somites) at once limits the range of defective embryos capable of development to those possessing a heart and vitelline circulation. Another limitation arises from the extreme sensitiveness of the embryo to removal of parts of the brain ; although I have made over seventy experiments on the brain, none of the embryos, in which the injury extended back of the optic stalks, has developed for more than about forty-eight hours after the operation. (The operations on the head form a class in themselves and will be discussed in a separate paper.) The reason for the large number of fatalities in operations in this region is probably not due to any trophic function of the nervous system, at least in stages younger than 72 hours, but either to direct injury to the anterior end of the heart, or to malformations of the amnion consequent on the operation. On the other hand, embryos may survive the destruction of a considerable portion of the posterior end, and develop normally for several days as least. All the defective embryos to be described resulted from operations of this kind, performed, with one exception, on embryos in which the tail-bud is just forming after 50–60 hours' incubation.

A larger or smaller part of the posterior end was destroyed by cauterization. The embryos might bear complete destruction of the posterior end up to the vitelline arteries, provided these were not injured, without any apparent detriment or hindrance to the development of the uninjured parts. If the vitelline arteries were destroyed, the embryo never survived. Fig. 1 gives a view of an embryo of about the age of those used for operations. In this case 29 somites are formed ; the number was certainly not over 30 at the hour of operation in any case. The somites are continued backward by the undivided segmental plate represent-

ing a considerable number of potential somites. As will be shown later, somites 26 to 32 are the ones that normally form the musculature of the leg. Thus the undivided mesoblast at this stage includes a large part of the trunk. The vitelline arteries at this time leave the embryo opposite to the twenty-first or twenty-second somites; this position appears to be very constant. Thus the theoretical limit of the experiments is from the hind end to about the twenty-second somite, because

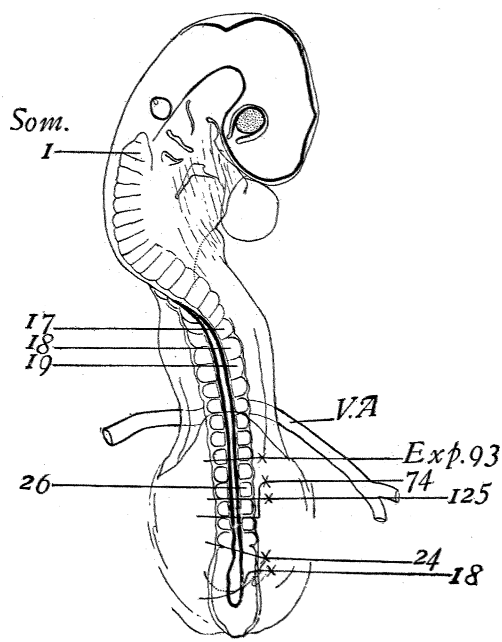

FIG. 1. Camera drawing of chick embryo with 29 somites; operation diagram. For explanation see text.

the embryo cannot live if the vitelline arteries are destroyed. The rudiment of the allantois lies beneath the unsegmented mesoblast beginning, approximately, a short distance back of the thirtieth somite.

Fig. 1 serves at the same time as a diagram of the operations. At the time of each operation a sketch was made of the embryo, and the part destroyed was indicated by shading the posterior end correspondingly. This sketch, naturally, did not show somites, so the present operation diagram is constructed from data deter-

mined after the operation. It agrees, however, fairly well with the original operation diagrams which were simply rough estimates of the amount destroyed by the operation. It should be distinctly understood that the figure is a result of study of the anatomy of the defective embryos. In further explanation it should be added, that the numbers to the right are the numbers of the experiments; those to the left of the somites. The lines leading to the numbers indicating the experiments are drawn across the body to indicate, that, in the experiment in question, all back of the line was destroyed; the cross on each line marks the junction of reference and operation lines.

A word concerning the enumeration of the somites; the somite numbered 1 possesses a short anterior process, that is probably an independent somite. It is, however, so inconspicuous in many embryos that it seemed better for the present purpose not to enumerate it. Somites 17, 18 and 19 are especially marked because they are the wing-somites, *i. e.*, the somites that will form the major portion of the bone and muscle of the wing. The twenty-sixth is the first leg somite. By this reckoning, there are three cephalic somites, or, reckoning in the incomplete one, four.

Referring to Fig. 1 again, it will be seen that the organs destroyed in such operations are: (1) The hind end of the neural tube; (2) the hind end of the notochord; (3) the mesoblastic segmental plate and often certain of the posterior mesoblastic somites; (4) the hind gut including the rudiment of the allantois; (5) the hind end of the Wolffian body and ducts.

I may say at once that no true regeneration of these structures takes place. (For discussion of this, see p. 50.) Therefore the problems of interest became narrowed down to the differentiation of the uninjured parts, and my attention has been particularly drawn to the behavior of the mesoblastic somites. In the somites we have an originally homonymous series of structures, the segmentation of which becomes strikingly heteronymous as development proceeds, some entering into the head, others the neck, others the wing, the thorax, abdomen, leg and tail. Definite somites, *i. e.*, somites in a definite numerical position in the series, form the skeleton and muscles of each of these regions. It would

be interesting to determine whether or not somites had the same rôle in defective as in normal embryos.

The numerical value of the somites in the chick seems to be normally as follows:

96-hour Chick.		Eight-day Chick.	
1, 2, 3,	Cephalic, hypoglossus region.[1]		
4 to 16,	Prebrachial, trunk.	1 to 13,	prebrachial.
17, 18, 19,	Brachial.	14, 15, 16,	brachial.
20 to 25,	Between wing and leg.	17 to 22,	between wing and leg.
26 to 32,	Leg somites.	23 to 29,	leg.
33 to 35,	Region of cloaca,	30 to 32,	region of cloaca.
36 to 42,	Caudal somites.	33 to 38,	caudal.

The count in the eight-day chick is really an enumeration of nerves. For the four-day chick the exact number of the leg somites was determined by comparison with the enumeration of nerves of the eight-day chick. The wing somites may readily be distinguished at four days, in entire mounts, by their size and by the relatively large amount of mesenchyme formed opposite them. In embryos of more than four days of age the enumeration by nerves is much the easier; and, as the brachial nerves (14, 15, 16) are very much larger than their neighbors (Figs. 3 and 5, *B.P.*), it is simplest in determining the place of a postbrachial somite or nerve to count only the somites back of the last brachial nerve. Thus the postbrachial somites 1–6 are between arm and leg; postbrachial 7–13 are leg-somites, etc.

Experiment 125.

In this experiment a very considerable part of the hind-end was destroyed at the time of appearance of the tail-bud (see operation-diagram), and the embryo was allowed to develop for about four days more (91 hours). The egg was then reopened. The vascular area covered at least three fourths of the yolk. There was no allantois. The embryo lay in large part beneath the blastoderm, but a large aperture in the latter towards the hind-end of the embryo was filled by the amnion through the transparent walls of which the embryo could be distinctly seen. A large part of the blastoderm was removed with the embryo and examination of the under surface was then made in salt solu-

[1] In this enumeration I have omitted again the incomplete anterior cephalic somite.

tion. The amnion was very well distended, and attached round the margins of the somatopleure at the hind-end. Behind this

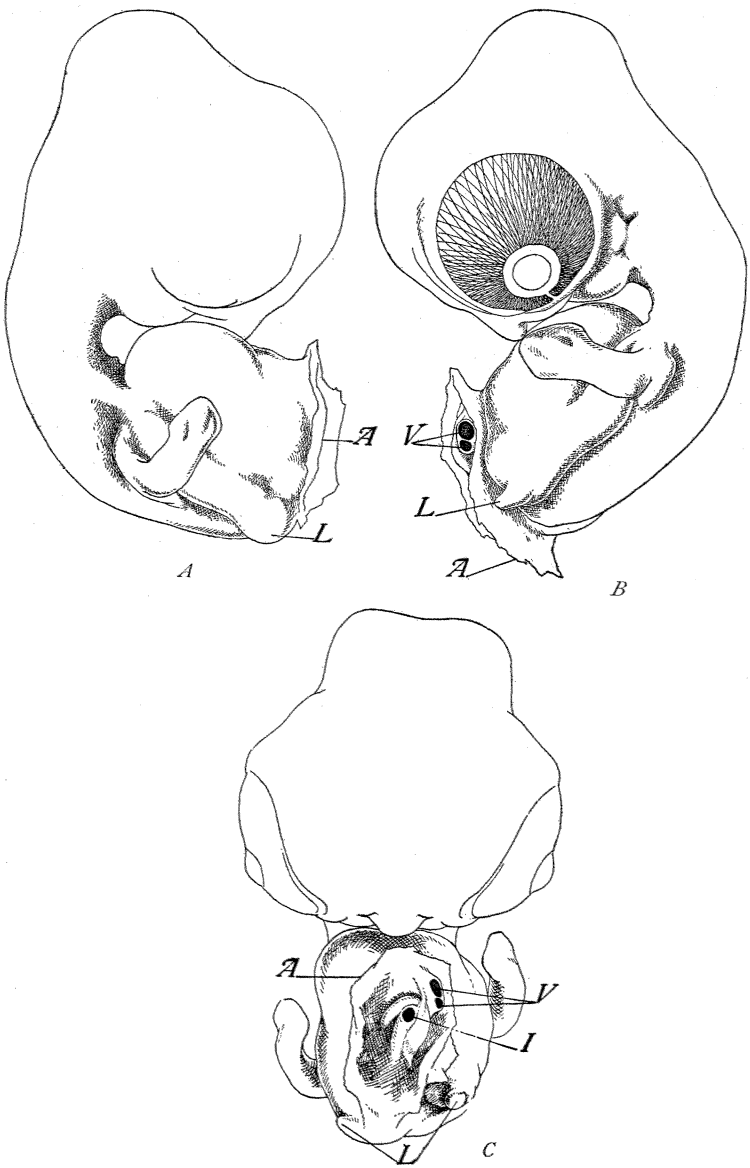

FIG. 2. Three views of a defective embryo (experiment 125) with membranes removed; total age about six days. *A*, amnion; *I*, ectopic intestine; *V*, vitelline artery and vein; *L*, rudiment of hind limbs.

the embryo was naked and there was slight ectopia of the intestine (Fig. 2, *C*).

Figures 2, *A*, *B* and *C* show this embryo from the two sides and from behind. The last figure shows especially well the attachment of the amnion and the ectopia of the intestine external to this. The hind-end of the embryo back of the vitelline vessels is wanting. On the other hand, all parts in front of this are normally developed.

On each side, at the hind-end of the Wolffian ridge, rudiments of the hind limbs are present as prominent conical stumps. From this it would appear at first sight that there has been some regeneration. However the question can be decided only by ascertaining the numerical value of the segments concerned in the formation of these stumps. The embryo was cut into sagittal sections for this purpose.

The enumeration of the post-brachial nerves is the same on both sides, seven pairs being present. The stumps are innervated only by the seventh on each side (Fig. 3). In the normal chick, the nerves innervating the leg are the seventh to the thirteenth postbrachial. Thus it would appear that only one leg somite on each side was uninjured by the operation, and this is the only one that has contributed to the formation of the rudimentary leg. None of the six somites between arm and leg has undergone any alteration of its normal numerical value. This result must therefore be interpreted in the sense of normal self-differentiation of the somites concerned.

Structure of the Leg-rudiments. — But, even though the deficiency of leg somites has not stimulated their immediate neighbors in front to any act of supererogation, it might be that the only leg somite remaining has produced more than its wont. In this connection the structure of the stumps is of interest: Owing to their positions, the left one is cut longitudinally and the right one transversely in the sagittal series. The structure is the same on the two sides (see Fig. 3); there is a single curved rod of precartilage extending out nearly to the tip of the limb; this is surrounded by a mass of dense mesenchyma, evidently premuscle tissue, and externally are the elements of the skin. Now, in the normal limb of corresponding age, the skeleton of the thigh,

crus and pes are separate chondrifications. The crus and pes are thus certainly not represented in the rudimentary hind-limb of this embryo.

In the normal chick the seventh postbrachial nerve innervates only some of the proximal muscles of the leg, those extending from the pelvis to the femur. On the principle that the muscular distribution of the nerve is confined to the derivatives of the corresponding somite, it follows that the seventh postbrachial somite contributes to the formation of only the upper segment of the leg.

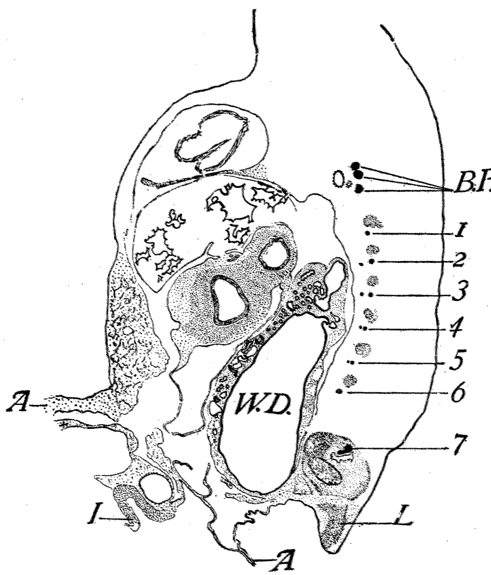

FIG. 3. Sagittal section of embryo shown in Fig. 2, cut considerably to the left of the middle line. *A*, amnion; *B.P.*, brachial plexus (nerves of wing); *I*, posterior end of intestine; *W.D.*, Wolffian duct; 1-7 first to seventh postbrachial nerves.

The absence of the crus and pes is therefore what might be expected if the principle of self-differentiation were rigidly adhered to. We have seen that, as a matter of fact, these are absent in the rudimentary hind limbs of this embryo, so we may conclude, at least, that there is no evidence that the seventh postbrachial somite has produced anything beyond the normal as a result of the absence of the following somites of the leg.

Alimentary Tract. — Practically all of the splanchnopleure of the embryo posterior to the vitelline arteries was destroyed by

the operation. In the resulting embryo, the trachea is a long tube, the lungs are budding out, the œsophagus is well formed, and the stomach is in two divisions, in the anterior of which the glands have begun to form, and the intestine is slightly coiled and opens on the hind surface, across which it is continued as a superficial strip ending in a free blind tag with a slight lumen (Figs. 2*C* and 3). The liver and pancreas are normally formed. In fact the parts present are apparently in the same condition that they would have been had the embryo been intact.

The allantois is entirely absent; and there is no evidence of any compensating growth of the intestine.

Nervous System. — The spinal cord ends bluntly, but the neural canal is prolonged backwards at its ventral angle into a short process which tapers into a bundle of neurites that may be followed a short distance, and then terminates abruptly. It would appear that the descending tracts in the cord have caused the prolongation, and have then pushed out into the adjacent tissues. A similar prolongation appears in all embryos defective at the hind-end (Fig. 3, *A*).

Excretory System. — The Wolffian ducts are much dilated, as there is no outlet for the secretion of the mesonephros (Fig. 3). No metanephric outgrowths have arisen from them, although in the normal embyro of this stage these are well developed. The metanephric region of the Wolffian ducts was of course destroyed by the operation; but, as the ducts are continuous structures, one would not anticipate that the capacity for producing metanephric outgrowths would be limited to a short stretch at the posterior end. Of course another explanation of their absence than that of limitation of potency to a short region of the Wolffian duct is possible, *i. e.*, that suitable predisposing external conditions for their formation are strictly limited (*e. g.*, that their development depends on a certain amount or kind of development of the allantois or cloaca).

Experiment 93.

In this experiment a very considerable portion of the hind-end was cauterized and removed (see operation diagram). Seventy-two hours later the egg was reopened and found living. The vascular area covered about three fifths of the yolk; the circulation was very active, and there was no allantois.

Fig. 4 is a view of the embryo in the amnion, drawn from the under side of the blastoderm. The vitelline blood vessels are attached to the center of the defective hind-end, the entire trunk back of these vessels being absent. The amnion is well distended; its line of attachment is indicated by the dotted line. Just behind this line of attachment is seen a little tag, the hind-end of the intestine. No trace of the hind-limbs is visible externally.

This embryo was cut into sagittal sections. In these there is no trace of the hind limbs, and no evidence that the posterior myotomes or sclerotomes are modified towards the formation of limbs. The embryo is not quite so far advanced in development

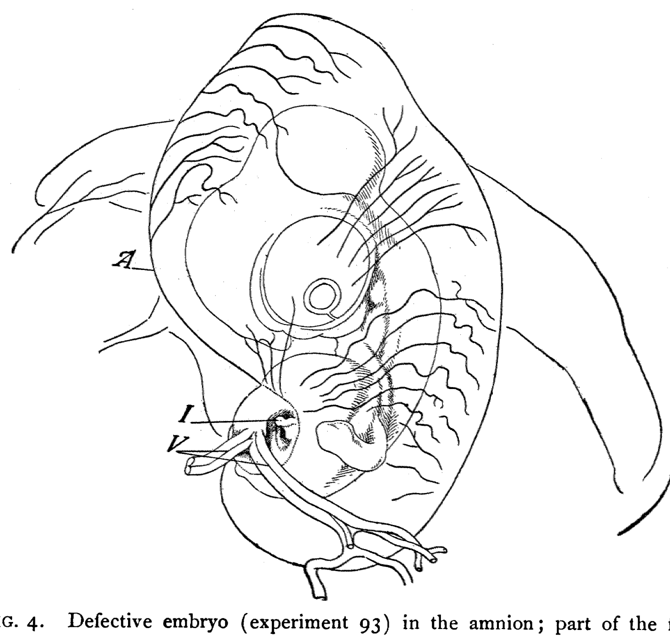

FIG. 4. Defective embryo (experiment 93) in the amnion; part of the folded under surface of the blastoderm is shown. *A*, amnion; *I*, ectopic intestine; *V*, vitelline arteries and veins.

as that of experiment 125, but it is certainly old enough to show the rudiments of the hind limbs or any part of them, were there any tendency towards their formation.

Study of the sections shows that there are only five pairs of post-brachial ganglia and nerves (Fig. 5), the fifth on both sides

being very small, and evidently partially destroyed by the operation. As the first leg-somite is the seventh post-brachial, the absence of rudiments of the hind limbs in this embryo is readily understood.

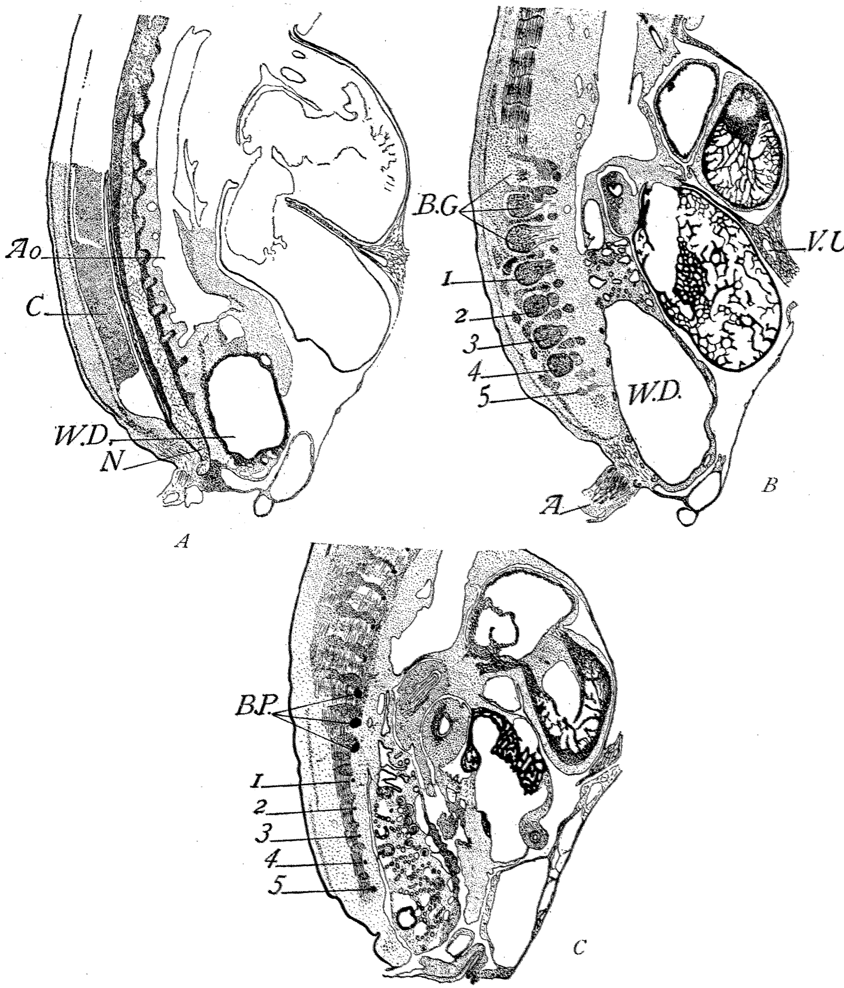

FIG. 5. Three sections from a sagittal series through the embryo shown in Fig. 4. *A*, amnion; *Ao.*, aorta; *B.G.*, ganglia of the nerves of the wing; *B.P.*, nerves of wing; *C*, cord; *N*, notochord; *V.U.*, umbilical vein; *W.D.*, Wolffian duct; 1-5, first to fifth postbrachial ganglia or nerves.

Thus there are two somites less in this embryo than in that of experiment 125. In the latter, which includes one leg somite, rudiments of the hind limbs are formed; in the present case no

such rudiments are formed, although the embryos are very much alike in every other way. It would be interesting to compare an embryo with six post-brachial somites, but I have none yet.

The central nervous system ends bluntly, except for a short prolongation of the ventral angle of the canal (Fig. 5, *A*). This condition, which is characteristic for all embryos defective at the hind-end, appears to be due to growth of fibers in the ventral motor zone, for a bunch of neurites extends back on each side of the ventral middle line nearly to the hind end of the notochord. The notochord extends backwards to the extreme posterior end, thus some distance further than the spinal cord. It thus appears, as in the other embryos of this class, to have undergone some regeneration, or at least growth, at the hind end.

The intestine opens at the posterior end, and is continued as a flat strip along the surface of the splanchnopleure. It is interesting to note that this strip has the same histological structure as the walls of the tube proper, showing that the histogenesis depends upon the character of the cells, and not upon such external factors as the form of the tube. The liver is normal. There is no trace of the allantois; but the stem of the umbilical veins may be seen, in a very rudimentary condition, running in the *septum transversum* to enter the anterior face of the liver (Fig. 5, *B*).

The *Wolffian bodies* are well developed, and the Wolffian duct much enlarged as in other anallantoic embryos (Fig. 5). Metanephric outgrowths are absent.

Summing up, we may say, that all the embryonic rudiments present have differentiated in the normal fashion. There has been no modification of the numerical value of the somites, no compensating growth, and no regeneration, unless we except the elongation of the notochord. Those embryonic organs, whose rudiment or ordinary locality was destroyed, are simply missing.

Experiment 74.

A large part of the hind end was destroyed at the stage of about 52 hours (see operation diagram). The egg was reopened about 68 hours after the operation. The vascular area covered fully half of the yolk; the heart-beat was vigorous.

Fig. 6 gives two views of the embryo. The hind end is entirely absent, and the opening is filled by the enlarged Wolffian ducts. The band of tissue between the two ducts is the hind end of the alimentary tract. The right side is seen to be less developed than the left. In the view from the opposite side, it can be seen that there is a rudiment of the hind-limb on the left side; but apparently none on the right.

This embryo was cut into transverse sections. Study of these shows that there are eight postbrachial ganglia on each side, though the eighth on the right side is smaller than on the left.

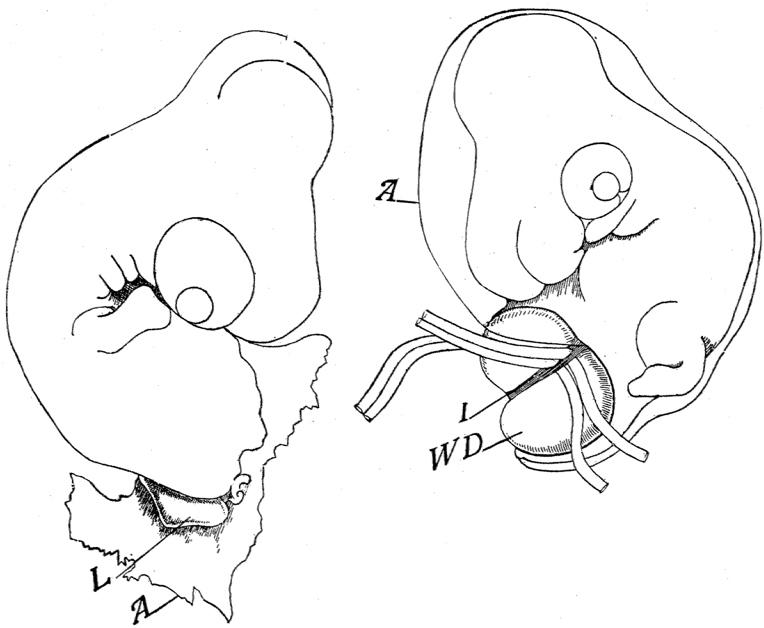

FIG. 6. Two views of a defective embryo (experiment 74). *A*, amnion; *I*, strip of intestinal epithelium; *L*, leg; *W.D.*, Wolffian duct. The vitelline blood vessels are somewhat abnormal.

On the left side the seventh and eighth postbrachial nerves enter the rudiment of the hind limb. On the right side there is no such rudiment, and the seventh and eighth nerves, which are quite large, end bluntly in the mesenchyme, almost as though cut off. The question arises why there is no limb rudiment on the right side? One can explain this by assuming that the operation destroyed the seventh and eighth postbrachial meso-

blastic somites on this side, but not the ganglia and cord of these somites (see Fig. 1). The form of the embryo indicates that this is true in part; but there is a small myotome in the eighth postbrachial somite of this side, and in the seventh the myotome is quite as well developed as on the other side. It would appear probable, then, that the most lateral portions of the seventh and eighth postbrachial mesoblastic somites were destroyed, together with the somatopleure lateral to this; either the rudiments of the hind limb were included in the destroyed parts, or their development was prevented by the scar-tissue. It is impossible to say whether or not the two somites concerned in the formation of the left leg have formed more than the normal.

The central nervous system terminates abruptly, except that the ventral portion of the canal is continued back as a narrow epithelial tube. The notochord extends some sections back of the termination of the neural tube, showing that it has been added to at its hind end since the operation.

The Wolffian ducts are immensely enlarged (see figures). There is no metanephric outgrowth. The allantois is entirely absent. The alimentary canal is normal back to the defective region and terminates in a band lying between the two Wolffian ducts (Fig. 6).

Experiment 18.

The tail-bud of the embryo was just formed at the time of the operation, and it was completely destroyed with a heated needle (see operation diagram). The injury did not extend so far forward as in the other cases mentioned and was asymmetrical (Fig. 1).

Fifty hours later the egg was reopened, and the embryo was found living, the heart beating actively; the vascular area covered about one third of the yolk.

Fig. 7 shows the embryo as it appeared from the under surface of the blastoderm. It will be observed that the amnion ceases with a free edge immediately in front of the hind-limbs; thus the tail-fold of the amnion destroyed by the operation has not regenerated, nor is the allantois visible externally, as it always is in normal embryos of this age. The hind-limbs appear normal; part of the tail at least is present.

The enumeration of the postbrachial nerves in the sections gives the following results : 1 to 6 on each side are in front of the leg-rudiments ; the following seven nerves on each side enter the leg-rudiments. On the right side there is only one incomplete ganglion back of the last leg-nerve, *i. e.*, the fourteenth postbrachial ; the nerve from this cannot be traced. On the left side there are four complete ganglia and myotomes back of the last leg nerve (14 to 17 postbrachial). Thus, as might be expected, the hind limbs are normal, and the cloacal region is strongly affected.

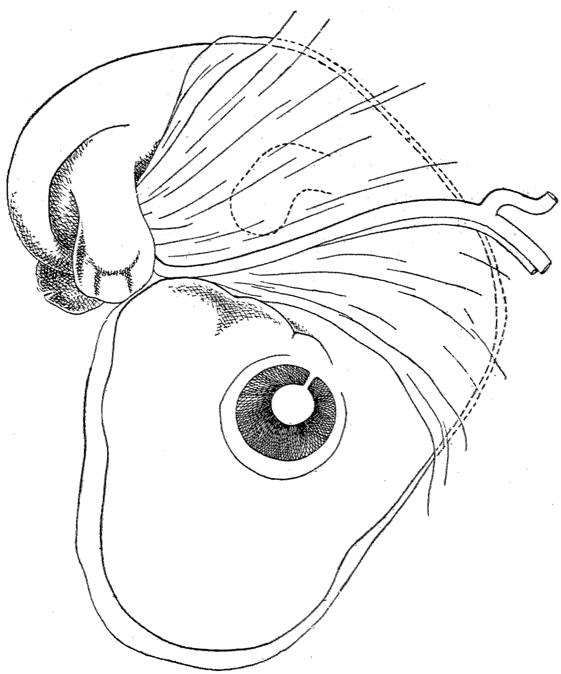

FIG. 7. Defective embryo (Experiment 18).

In the embryo of this experiment the Wolffian ducts have normal relations, but the allantois is thick-walled and undilated ; the entodermal cavity of the latter opens out into a groove in the splanchnopleure, leading back to the hind-gut, which is not closed as it normally is ; in fact in this embryo the cloaca is the only closed region of the hind-gut ; immediately in front of the cloaca it is open ventrally. The stalk of the allantois therefore appears first as a groove on the left side ; followed forwards, this

deepens gradually into a canal that penetrates into a mass of mesoblast connected with the somatopleure, and excavates a large irregular cavity in this. The mass extends forwards to the tip of the ventricle and ends in a bifurcated extremity. This represents the allantois, the main mass of which is composed of loose, very vascular mesenchyme forming an appendage to the somatopleure on the left side. On the right side the mesoblast of the somatopleure in the allantoic region is much hypertrophied and forms a large free lobe without any entodermal contents.

It is remarkable that the allantoic rudiments should show such considerable power of growth in the absence of the usual stimulus of internal pressure, which is precluded by the open groove-like connection with the intestine.

Experiment 24.

The hind end of an embryo was cauterized with the aim of destroying the incipient tail-bud and the region of the hind-

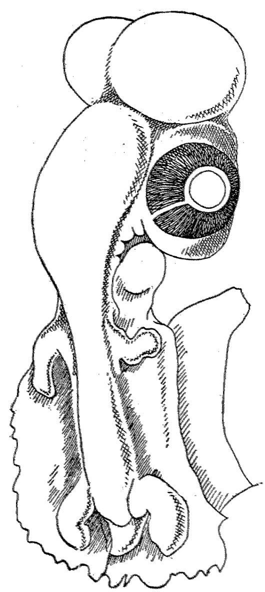

FIG. 8. Defective embryo (Experiment 24).

limbs. The egg was reopened 48 hours after the operation. The vascular area covered about two fifths of the yolk, and appeared to be entirely normal.

Fig. 8 represents the resulting embryo from the dorsal surface; the rudiments of the hind-limbs are apparently as well developed as those of the anterior limbs, and the posterior portion of the trunk is separated from the anterior by a sharp depression. The tail turns to the left, and tapers to an end beneath the left hind-limb; part of its course is hidden by a fold of the somatopleure, as shown in the figure. The depression evidently represents the anterior limit of the injury.

This embryo was sectioned. On examination it was found that, although the region behind the depression has the usual structure of the postanal region of the body, its neural tube has no connection with that of the trunk, and the notochord is absent in the anterior third. Mesoblastic somites occur in this region, but the main part of its substance is made up of loose vascular mesenchyme.

It is plain, then, that this part has not regenerated in the sense that it has grown out from the embryo; and there remains only the assumption that its parts have formed from embryonic tissue remaining in this region after the cauterization. The absence of the notochord throughout almost all of the tail shows that the destruction due to cauterization was very extensive; its absence beneath the neural tube for a considerable distance shows, that the neural tube has formed secondarily in this region; because any operation, that would destroy the notochord, would necessarily destroy the neural tube. Farther, the relation of the Wolffian ducts and allantois to the cloaca are abnormal, and the hinder portion of the intestine has no connection with the anterior portion; all of which shows that there was originally complete destruction of tissues through to the entoderm in the anterior part of the cauterized area. There seems to have been in this case a reorganization of the embryonic tissue of the caudal region.

On the left side I find ten postbrachial ganglia back to the region of the defect. Thus at least four segments of the normal hind limb-region are included, viz., 7, 8, 9, 10. The last is small, representing only a fraction of the normal ganglion. On the right side there are eleven full-sized postbrachial ganglia. Thus there are at least one and one half more metameres on the right than on the left side, and this tallies with larger size of the right hind limb and with the operation diagram.

The only interpretation of this result is that the needle destroyed a transverse section of tissue and separated the tail-rudiment from the rest of the embryo, at the same time causing some injury to the tissue of the tail; secondary union then took place. The tissue along the line of junction is peculiar in many respects, including much material, probably remnants of cauterized parts, that takes the plasma stain.

Experiment 187.

In this experiment the operation was made at a stage of about 24 hours; the medullary plate was just formed and the primitive streak was yet long; probably 3 to 5 somites were present (Fig. 9). Three spots were marked with an electrode, one behind the other in the middle line towards the posterior end of the primitive streak. The three spots were at first separate, but later examination showed them running together; so that all of the

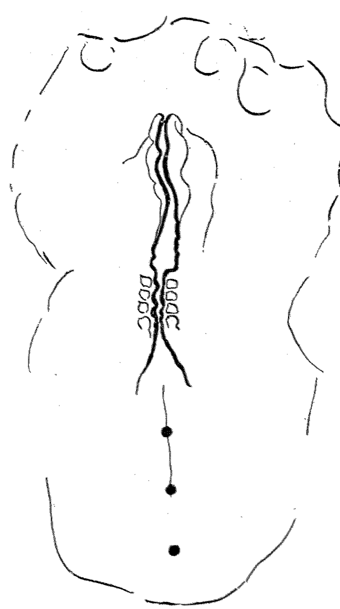

FIG. 9. Operation diagram for experiment 187; drawn from a blastoderm of the same lot of eggs used in this experiment; preserved at the time of the operation.

axial structures were destroyed in this region. The embryo was allowed to develop two days longer and was then preserved.

It will be seen (Fig. 10) that this operation has taken us in front of the vitelline arteries; having been performed before the development of the arteries, it was possible for the rudiment of the vascular system to readjust itself to the new conditions, and establish a vitelline circulation. The vitelline arteries are, however, in about their normal position, *i. e.*, about opposite the 20–22 somites, hence behind the embryo in this case. It is difficult to see why they should have this position and not be shifted farther forward, if the view of His, that the main blood vessels develop from the paths of least resistance in the primitive vascular network, be true.

On the right side of this embryo there are 14 spinal ganglia, and on the left 14 complete and part of the fifteenth; but as, in the fowl, the first two cervical nerves lack ganglia, there are present 16 spinal nerves on the right side, and 17 on the left.

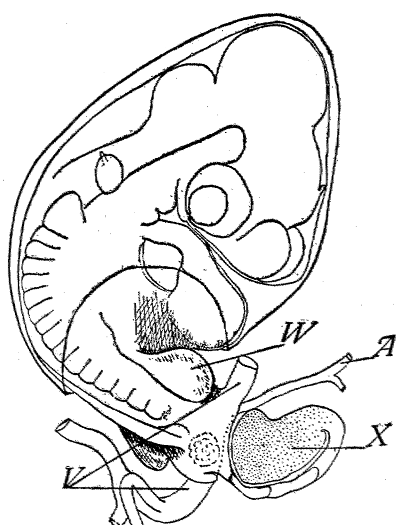

FIG. 10. Defective embryo (experiment 187). The drawing was made from the stained and cleared specimen. *A*, vitelline artery; *V*, vitelline veins; *W*, wing bud; *X*, sac filled with blood, behind the embryo in the prolongation of its axis; this has no connection with the vessels of the embryo.

The normal innervation of the wing is from the fourteenth to the sixteenth spinal nerves. It would thus appear, on the evidence of the nerves, that the operation has destroyed everything back of the wing somites. The wing-buds have a development that appears perfectly normal for this stage.

On the right side there are nineteen myotomes, and on the left twenty and a half. Fig. 10 shows the myotomes of the right side as they appeared in an entire mount of the stained embryo in oil; only 16 can be counted. But sections show, that the large anterior one is really three, and the nineteenth is represented by the mesoblast behind the last complete somite. If we reckon the three anterior somites as cephalic, this leaves sixteen trunk myotomes, and the enumeration agrees with that of the nerves. On the other side of the embryo there is distinctly one more cephalic myotome than on the right side; if, then, we reckon four as the number of cephalic myotomes on this side,[1] there are sixteen and a half trunk myotomes, a result that agrees with the enumeration of the nerves.

The organs in this embryo, as in the others described, have developed normally as far as the cut surface. The Wolffian ducts are enlarged by internal pressure. There has been no compensatory growth and no regeneration, if we except the notochord.

General Discussion.

The chief value of these experiments consists in the fact that they clear the ground for farther researches of a more definite character. It is probable that the results obtained will hold in general for the Amniota, and they may, therefore, be of value in the interpretation of teratological phenomena. Results that I have obtained from destruction of large portions of the brain in young embryos have convinced me that great caution must be exercised in interpreting conditions associated with anencephaly. It should be possible, and I am convinced that it is possible, to produce these various conditions experimentally. Until this is done it seems to me that conclusions, based on teratological material, as to the trophic value of the embryonic nervous system are debatable.

The following questions definitely raised by the experiments just described may be answered here in part:

1. *As to Regeneration.* — The only organ showing evidence of regeneration is the notochord. In all of the embryos described,

[1] Froricp recognizes four cephalic myotomes in the chick; the most anterior, however, becomes rudimentary at a very early stage, and soon disappears; this condition seems to have been attained on one side in this embryo, but not on the other.

the notochord extends a short distance back of the neural tube and its end is very protoplasmic; its further extension in each case is definitely limited by the surface of operation, against which it is pressed (Fig. 5, *A*). One receives the impression that its growth has been stopped by the mechanical hindrance.

One does not expect a vertebrate to show axial regeneration of the trunk, but in certain vertebrates the tail may regenerate. What is the condition in the chick embryo? No. 18 is the only case cited in which any of the caudal somites were left uninjured. In this case there was no regeneration, so far as could be judged.

The very first experiments that I performed on chick embryos were made to determine whether or not the limb-buds might regenerate. The operations were limited to the wing-buds. I found that it was possible to amputate the right one close to the body at a time when its width was equal to or greater than its length, and even later (four or five days); the wound in the amnion might close, and the amputated part remained in the amniotic cavity as evidence of the operation. In the only cases in which the embryo lived for any considerable period after the operation, there was absolutely no sign of regeneration, though the wing-bud of the opposite side increased in bulk several times.

So far as I know, the only evidence, that the organs of the chick embryo possess any different power of regeneration from those of the adult, consists of the above observations on the notochord (tissue regeneration) and of Barfurth and Dragendorff's[1] observation on the regeneration of the lens of the eye from the edge of the optic cup. The last depends on observations on a single case, and in this case the extent of injury to the eye was doubtful. Until the result is confirmed, I believe we are justified in passing it over.

There would remain, then, the general conclusion, subject only to the qualifications already noted, that the embryo of the chick possesses no greater power of regeneration than the adult.

2. *As to the Somites.* — Somites always retain their normal numerical value. But the experiments were not adapted to analyze very accurately the numerous problems presented.

[1] Dietrich Barfurth and O. Dragendorff, "Versuche über Regeneration des Auges und der Linse beim Hühnerembryo," Anat. Anz., Ergänzungsheft zum XXI. Bd., 1902.

Thus the operation destroyed in each case not only definite somites, but also all posterior to the first one injured, and the body wall lateral to the somites. With more refined methods, it may be possible to eliminate single somites from within the series, and to avoid injury to adjacent tissues. Such a technique would enable one to analyze many of the problems offered by the somites: to determine, for instance, the exact part played by them in development of the limbs, the order of origin of the most anterior somites, etc. Such problems are now being studied with partial success, and the results will be published later.

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